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May 05, 2000

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PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 C.F.R. 1.53(c).

Docket No. To be assigned		Type a plus sign (+) in this box	+

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TITLE OF THE INVENTION

STEREOSELECTIVE ANTIFIBRILLOGENIC PEPTIDES AND PEPTIDOMIMETICS THEREOF

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ENCLOSED APPLICATION PARTS (check all that apply)

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☒ Claims Number of Pages 4

☒Drawing(s) Number of Sheets 1 ☐ Other (specify)

METHOD OF PAYMENT (check one)

☐ A check or money order is enclosed to cover the Provisional filing fees	Provisional Filing Fee Amount $75.00 (Claiming Small Entity Status)
☒The Commissioner is hereby authorized to charge the filing fee to Deposit Account Number: 08-0219.	

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.
☒No
☐Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE W. A. K. DATE: May 5, 1999

TYPED or PRINTED NAME Wayne A. Keown, Ph.D. REGISTRATION NO: 33,923

☐ Additional inventors are being named on separately numbered sheets attached hereto

IN THE [redacted]ED STATES PATENT AND TRADEMA[redacted]FFICE

In re Applicati n of:	Robert Chalifour, Francine Gervais, Ajay K. Gupta
Serial No.:	TO BE ASSIGNED
Filing Date:	HEREWITH
D cket Number:	TO BE ASSIGNED
Title:	STEREOSELECTIVE ANTIFIBRILLOGENIC PEPTIDES AND PEPTIDOMIMETICS THEREOF

BOX PROVISIONAL PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

TRANSMITTAL LETTER

Enclosed herewith for filing in the United States Patent and Trademark office are the following documents:

1) Provisional Application Cover Sheet (one page);

2) Provisional Application (22 pages) with 21 pages of specification and 1 sheet of informal drawings comprising Figure 1;

3) Small Entity Statement (unsigned); and

4) Return postcard.

Respectfully submitted,

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CERTIFICATE OF MAILING

I hereby certify that the attached papers and fees are being deposited with the United States Postal Service as "Express Mail Post Office to Addressee" Service under 37 C.F.R. 1.10 on May 5, 1999 and is addressed to: BOX PROVISIONAL PATENT APPLICATION, Assistant Commissioner for Patents, Washington, D.C. 20231.

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STEREOSELECTIVE ANTIFIBRILLOGENIC PEPTIDES AND PEPTIDOMIMETICS THEREOF

BACKGROUND OF THE INVENTION

(a) Field of the Invention

The invention relates to agents having potent antifibrillogenic activity for the treatment of amyloidosis disorders and for imaging of amyloid plaque. These agents include peptides and peptidomimetic compounds thereof.

(b) Description of Prior Art

Amyloidosis refers to a pathological condition characterized by the presence of amyloid fibers. Amyloid is a generic term referring to a group of diverse but specific extracellular protein deposits which are seen in a number of different diseases. Though diverse in their occurrence, all amyloid deposits have common morphologic properties, stain with specific dyes (e.g. Congo red), and have a characteristic red-green birefringent appearance in polarized light after staining. They also share common ultrastructural features and common x-ray diffraction and infrared spectra.

Some amyloidotic diseases can be idiopathic but most of these diseases appear as a complication of a previously existing disorder. For example, primary amyloidosis can appear without any other pathology or can follow plasma cell dyscrasia or multiple myeloma. Secondary amyloidosis is usually seen associated with chronic infection (such as tuberculosis) or chronic inflammation (such as rheumatoid arthritis). A familial form of secondary amyloidosis is also seen in Familial Mediterranean Fever (FMF). This familial type of amyloidosis, as one of the other types of familial amyloidosis, is genetically inherited and is found in specific population groups. Isolated forms of

amyloidosis are those that tend to involve a single organ system. Different amyloids are also characterized by the type of protein present in the deposit. For example, neurodegenerative diseases such as scrapie, bovine spongiform encephalitis. Creutzfeldt-Jakob disease and the like are characterized by the appearance and accumulation of a protease-resistant form of a prion protein (referred to as AScr or PrP-27) in the central nervous system. Similarly, Alzheimer's disease, another neurodegenerative disorder, is characterized by congophilic cerebral angiopathy, neuritic plaques and neurofibrillary tangles. In this case, the plaque and blood vessel amyloid is formed by the deposition of fibrillar Aβ amyloid protein. Other systemic diseases such as adult-onset diabetes, complications of long-term hemodialysis and sequelae of long-standing inflammation or plasma cell dyscrasias are characterized by the accumulation of amyloids systemically. In each of these cases, a different amyloidogenic protein is involved in amyloid deposition.

Once these amyloids have formed, there is no known, widely accepted therapy or treatment which significantly dissolves the deposits in situ.

Each amyloidogenic protein has the ability to organize into β-sheet and to form insoluble fibrils which get deposited extracellularly. Each amyloidogenic protein, although different in amino acid sequence has the same property of forming fibrils and binding to other elements such as proteoglycan (glycosaminoglycan), amyloid P and complement component. Moreover, each amyloidogenic protein has amino acid sequences which, although different, will show similarities such as regions with the ability to

bind to GAG's (referred to as the GAG binding site) as well as other regions which will promote β-sheet formation.

In specific cases, amyloidotic fibrils once deposited, can become toxic to the surrounding cells. As per example, the Aβ fibrils organized as senile plaques have been shown to be associated with dead neuronal cells and microgliosis in patients with Alzheimer's disease. When tested in vitro, Aβ peptide was shown to be capable of triggering an activation process of the microglia (brain macrophages), which would explain the presence of microgliosis and brain inflammation found in the brain of patients with Alzheimer's disease.

In another type of amyloidosis seen in patients with Type II diabetes, the amyloidogenic protein IAPP, has been shown to induce β-islet cell toxicity in vitro. Hence, appearance of IAPP fibrils in the pancreas of Type II diabetic patients could contribute to the loss of the β islet cells (Langerhans) and organ dysfunction.

Particularly, in patients with Alzheimer's Disease, an agent capable of 1) preventing amyloid fibril formation and deposition and 2) of directly or indirectly being able to inhibit Aβ-induced neurotoxicity and inflammation (microgliosis), could be a treatment of choice to prevent and arrest the development of Alzheimer's disease.

It would be highly desirable to be provided with agents having potent antifibrillogenic activity for the treatment of amyloidosis disorders.

SUMMARY OF THE INVENTION

One aim of the present invention is to provide agents having potent antifibrillogenic activity for the treatment of amyloidosis disorders.

Another aim of the present invention is to provide a method for the treatment of amyloidosis disorders, such as Alzheimer's disease.

A number of strategies for possible therapeutic intervention in amyloid development have been proposed. These strategies include reduction of the pool of precursor proteins, prevention of the interaction of precursor proteins and disruption of preformed amyloid. The present invention deals mainly with the second approach, prevention of precursor protein interactions. The ideal molecule to fulfill this function, would interact specifically with the amyloid precursor protein and would in so doing, prevent the precursor from interacting with itself. When dealing with molecules which are chiral, it is standard practice to identify which of the stereoisomers possesses the activity, since in general, activity can be attributed to one or the other of the isomers. By using a stereochemically pure isomer, side reactions can be avoided or reduced.

In accordance with one embodiment of the present invention there is provided an antifibrillogenic agent for inhibiting amyloidosis and/or for neuroprotection, which comprises a peptide of Formula I, an L or D isomer thereof, a retro or a retro-inverso isomer thereof or a peptidomimetic thereof:

$$Xaa_1\text{-}Xaa_2\text{-}Xaa_3\text{-}Xaa_4 \quad I$$

wherein,

Xaa_1 is absent or selected from the group consisting of Lys, Lys-Lys, Xaa_5-Lys-;

Xaa_5 is absent or selected from the group consisting of His-Gln-, His-His-Gln-, Val-His-His-Gln-, Glu-Val-His-His-Gln-, Asp-Asp-Asp-, Lys-Val-Asp-Asp-Gln-Asp-;

Xaa_2 is absent or any amino acid;

Xaa_3 is absent, Val or Phe;

Xaa_4 is absent or selected from the group consisting of Phe, Phe-NH_2, Phe-Phe, Phe-Phe-Ala, Phe-Phe-Ala-NH_2, Phe-Phe-Ala-Gln, Phe-Phe-Ala-Gln-NH_2, Val-Leu-Lys, Val-Leu-Lys-NH_2;

wherein said peptide of formula I contains at least one Lys or Asp;

with the proviso that Lys-Lys-Leu-Val-Phe-Phe-Ala is an all-D peptide; and with the proviso that when Xaa_5 is Lys-Val-Asp-Asp-Gln-Asp- all of Xaa_2, Xaa_3, and Xaa_4.are absent.

In accordance with one embodiment of the present invention there is provided a labeled conjugate for in vivo imaging of amyloid plaque, which comprises a conjugate of formula I:

A-B-C

wherein A is a amyloid plaque-targeting compound selected from the group consisting of a peptide of Formula II, an L or D isomer thereof, a retro or a retro-inverso isomer thereof and a peptidomimetic thereof:

Xaa_1-Xaa_2-Xaa_3-Xaa_4 II

wherein,

Xaa_1 is absent or selected from the group consisting of Lys, Lys-Lys, Xaa_5-Lys-;

Xaa_5 is absent or selected from the group consisting of His-Gln-, His-His-Gln-, Val-His-His-Gln-, Glu-Val-His-His-Gln-, Asp-Asp-Asp-, Lys-Val-Asp-Asp-Gln-Asp-;

Xaa_2 is absent or any amino acid;
Xaa_3 is absent, Val or Phe;
Xaa_4 is absent or selected from the group consisting of Phe, Phe-NH_2, Phe-Phe, Phe-Phe-Ala, Phe-Phe-Ala-NH_2, Phe-Phe-Ala-Gln, Phe-Phe-Ala-Gln-NH_2, Val-Leu-Lys, Val-Leu-Lys-NH_2;
wherein said peptide of formula I contains at least one Lys or Asp;
with the proviso that Lys-Lys-Leu-Val-Phe-Phe-Ala is an all-D peptide; and with the proviso that when Xaa_5 is Lys-Val-Asp-Asp-Gln-Asp- all of Xaa_2, Xaa_3, and Xaa_4 are absent;
wherein B is a linker portion allowing attachment of the amyloid plaque-targeting compound to C;
wherein C is a label which allow for said imaging.

The preferred B moiety includes, without limitation, Glucose and Phe.

The preferred C moiety includes, without limitation, Tc and Re.

In accordance with the present invention, the preferred peptides of Formula I or II include, without limitation, D or L stereoisomer of peptides of the following amino acid sequences:

Lys-Ile-Val-Phe-Phe-Ala (SEQ ID NO:1)
Lys-Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:2)
Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:3)
Lys-Phe-Val-Phe-Phe-Ala (SEQ ID NO:4)
Ala-Phe-Phe-Val-Leu-Lys (SEQ ID NO:5)
Lys-Leu-Val-Phe (SEQ ID NO:6)
Lys-Ala-Val-Phe-Phe-Ala (SEQ ID NO:7)
Lys-Leu-Val-Phe-Phe (SEQ ID NO:8)
Lys-Val-Val-Phe-Phe-Ala (SEQ ID NO:9)
Lys-Ile-Val-Phe-Phe-Ala-NH_2 (SEQ ID NO:10)
Lys-Leu-Val-Phe-Phe-Ala-NH_2 (SEQ ID NO:11)
Lys-Phe-Val-Phe-Phe-Ala-NH_2 (SEQ ID NO:12)

Ala-Phe-Phe-Val-Leu-Lys-NH_2 (SEQ ID NO:13)
Lys-Leu-Val-Phe-NH_2 (SEQ ID NO:14)
Lys-Ala-Val-Phe-Phe-Ala-NH_2 (SEQ ID NO:15)
Lys-Leu-Val-Phe-Phe-NH_2 (SEQ ID NO:16)
Lys-Val-Val-Phe-Phe-Ala-NH_2 (SEQ ID NO:17)
Lys-Leu-Val-Phe-Phe-Ala-Gln (SEQ ID NO:18)
Lys-Leu-Val-Phe-Phe-Ala-Gln-NH_2 (SEQ ID NO:19)
His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-NH_2 (SEQ ID NO:20)
Asp-Asp-Asp (SEQ ID NO:21)
Lys-Val-Asp-Asp-Gln-Asp- (SEQ ID NO:22)
His-His-Gln-Lys (SEQ ID NO:23).

Other embodiments of these peptides include racemic mixtures and peptides having mixed chirality, i.e., different chirality at different chiral centers.

In accordance with the peptides Lys-Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:2)and Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:3), one stereoisomer, the D form, is found to be more active than the L form, and the D isomer is the preferred form for use of this peptide as a drug.

In accordance with the present invention there is provided a method for the treatment of amyloidosis disorders in a patient, which comprises administering to said patient a therapeutically effective amount of a peptide of Formula I or peptidomimetic thereof.

In accordance with the present invention there is provided a composition for the treatment of amyloidosis disorders in a patient, which comprises a therapeutically effective amount of a peptide of Formula I or peptidomimetic thereof in association with a pharmaceutically acceptable carrier.

In accordance with the present invention, the amyloidosis disorder includes, without limitation, prion protein related disorders and Alzheimer s disease. Other biological phenomenon are also

characterized by major participation of the GAG's. Chemokines are known to interact with the GAG's associated with cell surface proteoglycans. Specific chemokines require dimerization and tetramerization by associating with cell surface proteoglycan prior to their binding to their specific receptors. Diseases characterized by an uncontrolled chemokine response such as the acute respiratory distress syndrome, rheumatoid arthritis, etc, would benefit of a milder chemokine response obtained by the inhibition of the binding of chemokine to the GAG's leading to a decreased interaction between chemokine and their receptor.

For the purpose of the present invention the following expressions and terms are defined below.

The term "agents having stereoselective antifibrillogenic activity is intended to mean any peptides, peptide analogues, peptide derivatives, or peptidomimetics which retain the stereoselective antifibrillogenic activity, the neuroprotective and anti-inflammatory activity and/or the ability to alter natural Aβ (amyloidotic protein) aggregation as described herein. Peptide analogues, peptide derivatives, or peptidomimetics include any molecules which mimic the chemical structure of a peptide and retain the functional properties of the peptide. Examples of peptide analogues, peptide derivatives, or peptidomimetics including compounds with sulfonamide, phosphoramide or non-amide linkages.

The expression "antifibrillogenic activity" is intended to mean the ability to block or prevent an amyloidogenic protein from forming fibrils, preferably by preventing it from adopting its β-pleated conformation.

The term "neuroprotection" or "neuroprotective activity" is intended to mean the ability to protect cells from Aβ toxicity.

The term "anti-inflammatory" is intended to mean the ability to block or reduce the Aβ-induced microglial activation process or to block the chemokine-induced inflammatory reaction.

The term "retro isomer" is intended to mean a reversal of the direction of the peptide backbone.

The term "retro-inverso isomer" is intended to mean a reversal of both peptide backbone direction and an inversion of amino acid chirality.

The term "inverso isomer" is intended to mean an inversion of the amino acid chirality used to make the peptide.

Except as otherwise expressly defined herein, the abbreviations used herein for designating the amino acids and the protective groups are based on recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (Biochemistry, 1972, **11**:1726-1732).

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the targeted sites of the protein-protein interaction between the Aβ protein and the glycosaminoglycan moiety of the proteoglycan.

DETAILED DESCRIPTION OF THE INVENTION

As illustrated in Fig. 1, internal regions of the Aβ sequence have been shown to confer characteristics of the amyloid protein. Indeed, the region between amino acid 13-16 (HHQK) of the amyloid protein is responsible for the interaction between the Aβ protein and the glycosaminoglycan moiety of the proteoglycans (Kisilevsky, R., et al., Proteoglycans

and amyloid fibrillogenesis: The nature and origin of amyloid fibrils, Wiley, Chichester (CIBA Foundation Symposium 1997), pp. 58-72). Proteoglycans are known to promote amyloid fibril formation as well as protect these fibrils from proteolysis (Gupta-Bansal, R., et al., 1995, The Journal of Biological Chemistry, 270:18666-18671). More recently, the same region has been determined to play a role in the activation process of microglial cells by Aβ (Giulian, D., et al., 1998, The Journal of Biological Chemistry, 273(45):29719-29726). This 13-16 region of Aβ, often referred to as the GAG binding site, is also part of a larger domain, the 10-16 region of the protein which has been suggested as the region responsible for the adherence of the Aβ to cell surface (Giulian, D., et al., 1996, The Journal of Neuroscience, 16(19):6021-6037). Such adherence of Aβ to the cell surface will allow the interaction of Aβ with the specific cells leading to either microglia activation or toxicity of neuronal cells.

These two overlapping regions of the Aβ protein, i.e. amino acid 13-16 and 10-16 are adjacent to the 16-21 region of Aβ, a short hydrophobic stretch critical for the formation of fibrillar structures (Hilbrich, C., et al., 1992, J. Mol. Biol., 228:460-473). By having peptides capable of interacting with these overlapping regions of Aβ, one can aim at preventing both Aβ fibril formation and Aβ cellular interaction (i.e. microglia activation, neurotoxicity).

A preferred embodiment of the present invention is novel and arises from the unexpected finding that the all-D stereoisomer peptides, Lys-Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:2) and Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:3), are much more potent inhibitors of Aβ(1-40)

fibrillogenesis then the corresponding all-L peptides. The all-D stereoisomer peptides, Lys-Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:2) and Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:3) are also potent neuroprotective agents.

This finding was unforeseen particularly because the researchers who originally reported peptides containing the sequence Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:3) as an inhibitor of fibrillogenesis, state in a second article which they published: "A peptide entirely composed of amino acids in D configuration with the sequence klvff (lowercase marks amino acids in D configuration) was synthesized using the SPOT technique and assayed for ^{125}I-LBMP1620 binding. This peptide failed to bind ^{125}I-LBMP1620 (data not shown) indicating that KLVFF-KLVFF interaction is sterospecific." Tjernberg, L.O. et al. (1997) Controlling Amyloid β-Peptide Fibril Formation with Protease-stable Ligands, J. Biol. Chem, 272:12602.

The experimental work performed leading to this invention included comparing the ability of the d and l stereoisomers of peptide Lys-Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:2) to inhibit the fibrillogenesis process observed with the amyloidogenic peptide Aβ(1-40) in two assays, the Thioflavin T fluorescence assay and NBD-Aβ fluorescence assay.

The thioflavin T fluorescent assay for fibrillogenesis is based on the principle that the fluorescent dye, thioflavin T, binds specifically to fibrillar, but not to unaggregate Aβ peptide (LeVine III, H., 1993, Protein Science 2:404-410). Upon binding, thioflavin T develops a characteristic fluorescence (Naiki, H., et al., 1996, Lab. Invest. 74: 374-383) which can be easily detected. The dye is believed to interact with the stacked cross-β pleated

sheets, the common structural motif of all amyloid (LeVine III, H., 1995, Amyloid: Int. J. Exp. Clin Invest. 2:1.6). Thioflavin T is widely used to assay the effect of compounds on Aβ peptide fibrillogenesis (Bronfman, P.C., et al., 1995, Neuroscience Lett. 218:201-203).

In this assay test compounds are incubated with a solution of Aβ(1-40) (20 μM) containing 10 μM Thioflavin T, in 0.02M Tris/0.02M acetate/0.15M NaCl/0.005% azide/pH 7.40 at 37°C in sealed 384 well microplates. Readings (ex 430 nm/em 485nm) are taken at various time intervals with a microplate fluorescence reader. An increase in fluorescence signifies the appearance of amyloid or intermediates in the production of amyloid.

The results illustrated in Table 1 below, are based on the fluorescence production in the presence of test peptides at either 20 μM or 80 μM concentration, at the time intervals of 5, 19, 45, 67, 77 and 90 hours, compared to a control, buffer alone, without added inhibitory peptide.

Table 1

Order Of Potency of Peptide Inhibitors

	Tested at 20 M	Tested at 80 M
(strongest activity)	1 (D) KIVFFA	1 (D) AFFVLK
	2 (D) KKLVFFA	1 (D) KKLVFFA
	3 (D) KLVFFA	1 (D) KLVFFA
	4 (D) KFVFFA	1 (D) KFVFFA
	5 (D) AFFVLK	5 (D) KIVFFA
	6 (D) KLVF	6 (D) KAVFFA
	7 (D) KAVFFA	7 (L) KKLVFFA
	8 (L) KLVFFA	8 (L) KLVFFA
	9 (D) KLVFF	9 (D) KLVF

	10 (L) KKLVFFA	10 (D) KLVFF
(least activity)	11 (L) AFFVLK	11 (L) AFFVLK

Protocol

Aβ peptide: Aβ(1-40) 95% purity (American Peptide Company, Inc, Sunnyvale, Cal. USA, cat. 62-0-78) is disaggregated in trifluoroacetic acid and filtered through a 0.02 μM filter, (Whatman Anotop 25 plus, .02 m, Catalogue no. 6809 4102 in hexafluoroisopropanol (HFIP). Solutions of Aβ(1-40) at 600 m in HFIP are stored at -80C.

Assay mixture: The mixture is prepared as two solutions which are combined upon addition to the 384 well microplate (Corning Costar cat. 3705).

i) Solution A consists of test peptides in 0.02M Tris/0.02M acetate/0.15M NaCl/0.01 % azide at pH 7.40 or buffer alone (control),

ii) Solution B consists of Aβ(1-40) 40 M, Thioflavin T 20 μM in 0.02M Tris/0.02M acetate/0.15M NaCl at pH 7.40. This solution is prepared by drying the Aβ peptide under nitrogen and then resuspending this in 0.04M Tris base with 15 minutes sonication. An equal volume of 0.04M acetic acid containing 0.3 M NaCl is added and the solution is adjusted to 7.40±0.02. A small volume of 5mM Thioflavin T is added to the solution to give a final 20 μM concentration of Thioflavin T.

iii) The microplate is loaded with 40 μL of solution A followed by 40 μL of solution B which gives a final 20 μM Aβ(1 -40), 10 μM Thioflavin T, and either 20 μM or 80 μM test compound in 0.02M Tris/0.02M acetate/0.15M NaCl/0.005% azide, pH 7.40. The plate is sealed and loaded into the microplate fluorescence reader.

Fluorescence measurement data analysis: The HTS-7000 Bio Assay Reader, Perkin Elmer, is used to perform kinetic runs of about 5 days. Readings were taken at various time intervals, 5, 19, 45, 67, 77 and 90 hours, with one minute shaking before each read. Bandpass filters used were: excitation 430 nm, emission 485 mm.

Calculations

The rank order of efficacy of the peptides is determined by observing which peptides allow the appearance of fluorescence, above the background level, first. For example in the presence of buffer control alone, fluorescence appears earlier than when any of the peptides is present. The most active peptides prevent the appearance of fluorescence even after 90 hours of incubation.

The results achieved in the Thioflavin T fibrillogenesis assays show that all-D stereoisomer peptide was about 60 times more potent then the all-L stereoisomer peptide. This estimate is based on the observation that 400 uM all-L stereoisomer was required to give an equivalent inhibition to that produced with 6.1uM all-D stereoisomer peptide.

The results achieved in the Aβ-NBD environmental probe fibrillogenesis assay showed that the all-D stereoisomer peptide was at least 30 times more potent than the all-L stereoisomer peptide. This estimate is based on the observation that the lowest concentration of all-D peptide tested (25 uM) was more potent than the highest concentration of the all-L peptide (800 uM).

GAG binding domain peptide

Novel peptides and peptidomimetics that include complementary sequences to certain portion of amyloidogenic peptides such as Aβ, AA, AL, IAPP, prion

proteins and chemokines such as IL-8, Rantes, Eotaxin are designed to be capable of inhibition of Protein-Protein interactions or self assembly. The targeted portions in the various diseases causing proteins aforementioned, preferably contain one or more charged residues such as aspartate, glutamate, lysine, histidine and arginine. It is believed that such peptides and their peptidomimetics will inhibit fibrillogenesis of the amyloidogenic peptides and prion proteins and interfere with chemokines binding to the cell surface proteoglycans leading to dimerization or tetramerization by interacting with their GAG binding domains. In the case of Aβ, these interactions may lead to neuroprotection as well as inhibition of inflammatory response and serve as potent therapeutics for the treatment of Alzheimer's disease. In the case of chemokine-related disorder these interactions may lead to a decrease in the uncontrolled inflammatory response associated with some diseases.

Novel peptides containing 3-6 residues that are complementary (in terms of their charges) to the 10-16 segment of Aβ peptide have been shown for the first time to strongly interact with Aβ peptide. They provide a starting point for the design of BBB permeable peptidomimetics. In principle, similar peptides can be designed for the other amyloidogenic peptides such as AA, AL, IAPP and chemokines such as IL-8, Rantes, Eotaxin, etc.

Asp-Asp-Asp (SEQ ID NO:9), a tripeptide, when incubated with Aβ40 under physiological conditions shows a slight decrease at time t=0 in the amount of β-sheet content as is evident by the CD spectrum. Incubation of this tripeptide with Aβ40 for 24 hours shows no trace of β-sheet conformation of the Aβ40 and

clearly indicates the ability of this tripeptide to strongly interact with Aβ40 peptide and keep Aβ40 in a randomized and non-fibrillary conformation. The anti-fibrillogenic property of this tripeptide is also supported by the Aβ42 solubilization assay.

Lys-Val-Asp-Asp-Gln-Asp (SEQ ID NO:10), a hexapeptide, when incubated with Aβ40 under physiological conditions shows an increase at time t=0 in the amount of β-sheet content as is evident by the CD spectrum. Incubation of this hexapeptide with Aβ40 for 24 hours shows a dramatic increase in β-sheet content of the Aβ40 and clearly indicates the ability of this hexapeptide to strongly interact with Aβ40 peptide and organize it into a β-sheet conformation. The Electron microscopy of the mixture failed to show any fibrils indicating that this particular compound is in fact an anti-fibrillogenic compound with regard to Abeta. In vitro results with NBD and Thioflavin-T based fluorescence assays confirm this finding. It is the understanding of the discoverer that this interesting observation will lead to a greater understanding of fibrillogenesis of Aβ40 and Aβ42 peptides and as a result, will provide important information for the design of potent anti-fibrillogenic compounds for Aβ, other amyloidotic peptides such as AA, AL and IAPP for the treatment of diseases such as Alzheimer's, Type II Diabetes and amyloidosis related disorders. The same principle can also be applied to the design of peptide type compounds for the inhibition of binding of various chemokines to the cell surface as well as inhibition of self assembly of prion proteins.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications

and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

WHAT IS CLAIMED IS:

1. An antifibrillogenic agent for inhibiting amyloidosis and/or for neuroprotection, which comprises a peptide of Formula I, an L or D isomer thereof, a retro or a retro-inverso isomer thereof or a peptidomimetic thereof:

$$Xaa_1\text{-}Xaa_2\text{-}Xaa_3\text{-}Xaa_4 \quad I$$

wherein,

Xaa_1 is absent or selected from the group consisting of Lys, Lys-Lys, Xaa_5-Lys-;

Xaa_5 is absent or selected from the group consisting of His-Gln-, His-His-Gln-, Val-His-His-Gln-, Glu-Val-His-His-Gln-, Asp-Asp-Asp-, Lys-Val-Asp-Asp-Gln-Asp-;

Xaa_2 is absent or any amino acid;

Xaa_3 is absent, Val or Phe;

Xaa_4 is absent or selected from the group consisting of Phe, Phe-NH_2, Phe-Phe, Phe-Phe-Ala, Phe-Phe-Ala-NH_2, Phe-Phe-Ala-Gln, Phe-Phe-Ala-Gln-NH_2, Val-Leu-Lys, Val-Leu-Lys-NH_2;

wherein said peptide of formula I contains at least one Lys or Asp;

with the proviso that Lys-Lys-Leu-Val-Phe-Phe-Ala is an all-D peptide; and with the proviso that when Xaa_5 is Lys-Val-Asp-Asp-Gln-Asp- all of Xaa_2, Xaa_3, and Xaa_4.are absent.

2. The antifibrillogenic agent of claim 1, wherein said peptide of Formula I is selected from the group consisting of:

Lys-Ile-Val-Phe-Phe-Ala (SEQ ID NO:1)

Lys-Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:2)

Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:3)

Lys-Phe-Val-Phe-Phe-Ala (SEQ ID NO:4)

Ala-Phe-Phe-Val-Leu-Lys (SEQ ID NO:5)

Lys-Leu-Val-Phe (SEQ ID NO:6)

Lys-Ala-Val-Phe-Phe-Ala (SEQ ID NO:7)
Lys-Leu-Val-Phe-Phe (SEQ ID NO:8)
Lys-Val-Val-Phe-Phe-Ala (SEQ ID NO:9)
Lys-Ile-Val-Phe-Phe-Ala-NH_2 (SEQ ID NO:10)
Lys-Leu-Val-Phe-Phe-Ala-NH_2 (SEQ ID NO:11)
Lys-Phe-Val-Phe-Phe-Ala-NH_2 (SEQ ID NO:12)
Ala-Phe-Phe-Val-Leu-Lys-NH_2 (SEQ ID NO:13)
Lys-Leu-Val-Phe-NH_2 (SEQ ID NO:14)
Lys-Ala-Val-Phe-Phe-Ala-NH_2 (SEQ ID NO:15)
Lys-Leu-Val-Phe-Phe-NH_2 (SEQ ID NO:16)
Lys-Val-Val-Phe-Phe-Ala-NH_2 (SEQ ID NO:17)
Lys-Leu-Val-Phe-Phe-Ala-Gln (SEQ ID NO:18)
Lys-Leu-Val-Phe-Phe-Ala-Gln-NH_2 (SEQ ID NO:19)
His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-NH_2 (SEQ ID NO:20)
Asp-Asp-Asp (SEQ ID NO:21)
Lys-Val-Asp-Asp-Gln-Asp- (SEQ ID NO:22)
His-His-Gln-Lys (SEQ ID NO:23).

3. A labeled conjugate for in vivo imaging of amyloid plaque, which comprises a conjugate of formula I:

A-B-C

wherein A is a amyloid plaque-targeting compound selected from the group consiting of a peptide of Formula II, a L or D isomer thereof, a retro or a retro-inverso isomer thereof and a peptidomimetic thereof:

Xaa_1-Xaa_2-Xaa_3-Xaa_4 II

wherein,
Xaa_1 is absent or selected from the group consisting of Lys, Lys-Lys, Xaa_5-Lys-;
Xaa_5 is absent or selected from the group consisting of His-Gln-, His-His-Gln-, Val-His-His-Gln-, Glu-Val-His-His-Gln-, Asp-Asp-Asp-, Lys-Val-Asp-Asp-Gln-Asp-;
Xaa_2 is absent or any amino acid;

Xaa_3 is absent, Val or Phe;
Xaa_4 is absent or selected from the group consisting of Phe, Phe-NH_2, Phe-Phe, Phe-Phe-Ala, Phe-Phe-Ala-NH_2, Phe-Phe-Ala-Gln, Phe-Phe-Ala-Gln-NH_2, Val-Leu-Lys, Val-Leu-Lys-NH_2;
wherein said peptide of formula I contains at least one Lys or Asp;
with the proviso that Lys-Lys-Leu-Val-Phe-Phe-Ala is an all-D peptide; and with the proviso that when Xaa_5 is Lys-Val-Asp-Asp-Gln-Asp- all of Xaa_2, Xaa_3, and Xaa_4.are absent;
wherein B is a linker portion allowing attachment of the amyloid plaque-targeting compound to C;
wherein C is a label which allow for said imaging.

4. The labeled conjugate of claim 3, wherein said peptide of Formula II is selected from the group consisting of:
Lys-Ile-Val-Phe-Phe-Ala (SEQ ID NO:1)
Lys-Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:2)
Lys-Leu-Val-Phe-Phe-Ala (SEQ ID NO:3)
Lys-Phe-Val-Phe-Phe-Ala (SEQ ID NO;4)
Ala-Phe-Phe-Val-Leu-Lys (SEQ ID NO:5)
Lys-Leu-Val-Phe (SEQ ID NO:6)
Lys-Ala-Val-Phe-Phe-Ala (SEQ ID NO:7)
Lys-Leu-Val-Phe-Phe (SEQ ID NO:8)
Lys-Val-Val-Phe-Phe-Ala (SEQ ID NO:9)
Lys-Ile-Val-Phe-Phe-Ala-NH_2 (SEQ ID NO:10)
Lys-Leu-Val-Phe-Phe-Ala-NH_2 (SEQ ID NO:11)
Lys-Phe-Val-Phe-Phe-Ala-NH_2 (SEQ ID NO:12)
Ala-Phe-Phe-Val-Leu-Lys-NH_2 (SEQ ID NO:13)
Lys-Leu-Val-Phe-NH_2 (SEQ ID NO:14)
Lys-Ala-Val-Phe-Phe-Ala-NH_2 (SEQ ID NO:15)
Lys-Leu-Val-Phe-Phe-NH_2 (SEQ ID NO:16)
Lys-Val-Val-Phe-Phe-Ala-NH_2 (SEQ ID NO:17)

Lys-Leu-Val-Phe-Phe-Ala-Gln (SEQ ID NO:18)
Lys-Leu-Val-Phe-Phe-Ala-Gln-NH_2 (SEQ ID NO:19)
His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-NH_2 (SEQ ID NO:20)
Asp-Asp-Asp (SEQ ID NO:21)
Lys-Val-Asp-Asp-Gln-Asp- (SEQ ID NO:22)
His-His-Gln-Lys (SEQ ID NO:23).

5. The labeled conjugate of claim 3, wherein B is selected from the group consisting of Glucose and Phe.

6. The labeled conjugate of claim 5, wherein C is selected from the group consisting of Tc and Re.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No.: To Be Assigned)

Applicant or Patentee: Robert Chalifour *et al.*

Serial No. TO BE ASSIGNED

Filed or Issued: TO BE ASSIGNED

For: STEREOSELECTIVE ANTIFIBRILLOGENIC PEPTIDES AND PEPTIDOMIMETICS THEREOF

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 C.F.R. § 1.9(f) AND § 1.27(c)) - SMALL BUSINESS CONCERN

I hereby declare that I am an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF SMALL BUSINESS CONCERN: Neurochem
ADDRESS OF SMALL BUSINESS CONCERN: 7220 Frederick-Banting, Suite 100, Montreal, Quebec H4S 2A1, Canada

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 C.F.R. § 121.12 and reproduced in 37 C.F.R. § 1.9(d), for purposes of paying reduced fees to the United States Patent and Trademark Office, in that the number of employees of the business concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third part or parties controls or has the power to control both.

I hereby declare that the rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled

STEREOSELECTIVE ANTIFIBRILLOGENIC PEPTIDES AND PEPTIDOMIMETICS THEREOF

by the inventors, Robert Chalifour, Francine Gervais, and Ajay K. Gupta, described in

[X] the specification filed herewith

[ ] Application Serial No._____, filed _____.

[ ] Patent No. _____, issued _____.

If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 C.F.R. § 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 C.F.R. § 1.9(d), or a nonprofit organization under 37 C.F.R. § 1.9(e).

FULL NAME _____

ADDRESS _____

[ ] Individual [ ] Small Business Concern [ ] Nonprofit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 C.F.R. § 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief ar believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing therein, or any patent to which this verified statement is directed.

_____

NAME OF PERSON SIGNING

_____

TITLE IN ORGANIZATION

_____

ADDRESS OF PERSON SIGNING

_____

Signature

Date: _____

Neurochem Inc.

Protein - Protein Interaction: Targetted Sites

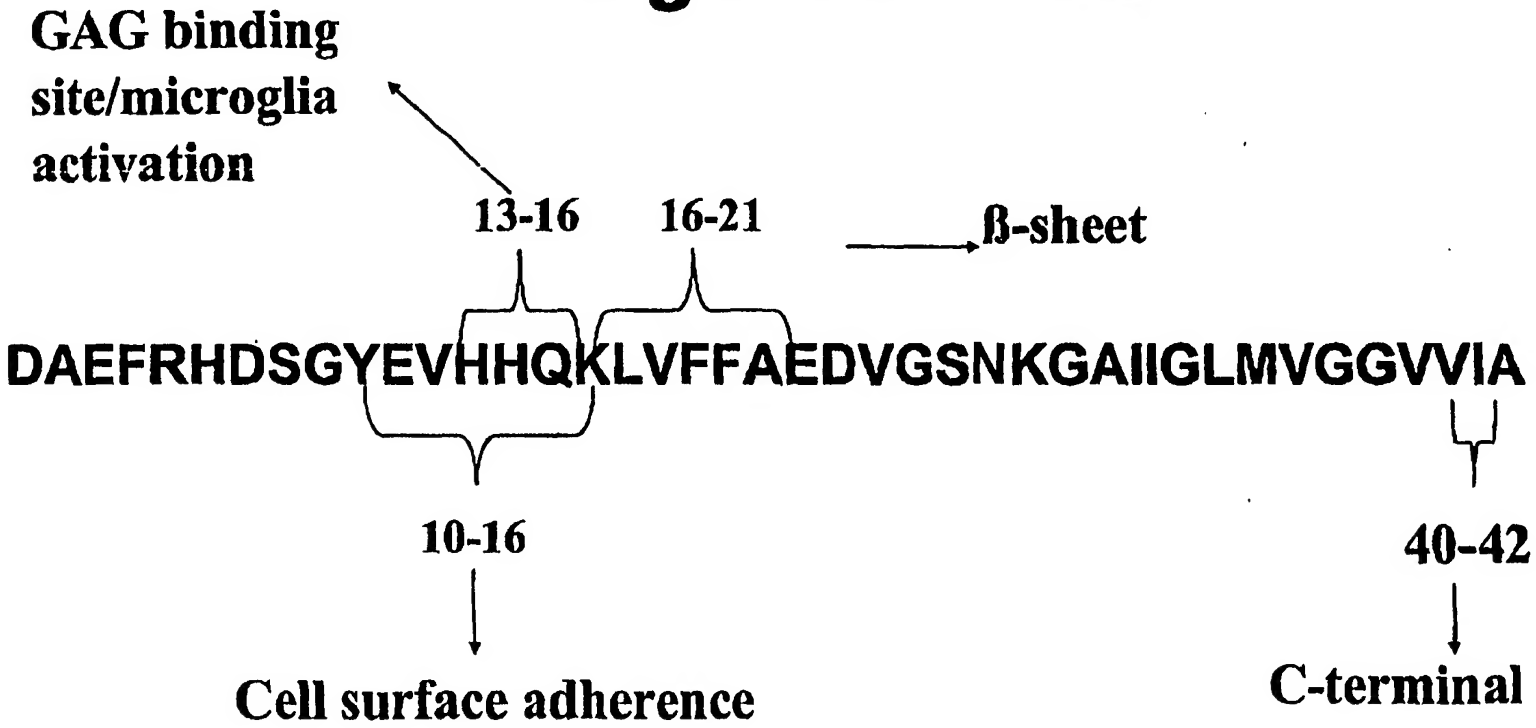

Fig. 1